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BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS | BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE | CHAMBRES DE RECOURS D L'OFFICE EUROPEEN DES BREVET

DECISION of 27 January 1985

Case Number:	T 0292/85
Boards of appeal:	3.3.
Application Number:	78300596.0
IPC:	C12K001/02
Language of the proceedings:	EN
Published in OJ:	1989/275
Cited in the CLBA:	CLBA 1996 ; OJ/EPO-SE 1997
Published on CD-ROM LEGAL:	1993/002

Applicant:

Genentech

Headword:

Polypeptide expression

Articles & Rules:

EPC Art 056, EPC Art 083, EPC Art 084, EPC Art 123, EPC R 027(1)(f)

Keyword:

Sufficiency of disclosure

Functional terms in claims

Provision of all embodiments

Inclusion of future inventions

Non-availability or unsuitability of some components

Non-availability of starting materials for general processes

Inventive step

Closest art pointing away from invention

Trial doomed to failure

Common general knowledge

Publications qualifying in early history of a particular art

Non-obviousness of plasmids

Other claimed subject-matter involving plasmids

Headnote:

1. An invention (here: biological) is sufficiently disclosed if at least one way is clearly indicated enabling th person skilled in the art to carry out the invention. Then the non-availability of some particular variants o unsuitability of some unspecified variants of a functionally defined component feature of the invention is immateria to sufficiency as long as there are suitable variants known to the skilled person through the disclosure or commo general knowledge which provide the same effect for the invention. The disclosure need not include specifi instructions as to how all possible component variants within the functional definition should be obtained (cf. poin

3.1.5 of the Reasons).

2. Generally applicable biological processes are not insufficiently described for the sole reason that some startin materials or genetic precursors therefor, e.g. a particular DNA or plasmid, are not readily available to obtain eac and every variant of the expected result of the invention (here: product) provided the process as such i reproducible (cf. point 3.3.3 of the Reasons).

3. The non-obviousness of the plasmids also imparts an inventive step to the other claimed subject-matters relating t their preparation and to their use for making polypeptides and immunogenic substances.

Summary of Facts and Submissions

I. European patent application 78 300 596.0, filed on 6 November 1978 and published on 16 May 1979 with publicatio number 1929, was refused by the decision of the Examining Division of the European Patent Office dated 15 May 198 and notified on 23 July 1985. The decision was based on Claims 1 to 12. The main Claims 1 and 9 were worded a follows: "1. A recombinant plasmid suited for transformation of a bacterial host comprising a homologous regulon an heterologous DNA, the heterologous DNA encoding a functional heterologous polypeptide or intermediate therefor, sai homologous regulon being arranged with said heterologous DNA so as to control transcription and translation of sai heterologous DNA encoding said functional heterologous polypeptide or intermediate therefor, whereby on translation o the transcription product of the heterologous DNA in a suitable bacterium, the resulting expression product is sai functional polypeptide or intermediate therefor in recoverable form

9. A bacterium transformed with a cloning vehicle according to any one of Claims 1 to 7."

II. The stated grounds for the refusal were that the disclosure was not sufficient under Article 83 EPC, includin questions arising from Rule 27(1)(f) EPC, there was consequently a lack of proper **support** under Article 84 EPC and no inventive step could be recognised under Article 56 EPC over the reference Polisky et al. (Proc. Natl. Acad Sci. USA, 1976, 73, 3900-3904) (1). The Examining Division insisted that all embodiments in the claims must have bee capable of being carried out by the skilled person at the priority date and in a repeatable manner without practisin inventive skill. No claims should rely on constituents which represent further inventions. In addition to th impossibility of providing such embodiments at the present, the later patentability of such constituent variants migh be adversely affected. Claims should, in effect, at least be limited to what is available at the priority date, i.e known bacteria, plasmids and DNA relating to known polypeptides. A process for the preparation of a human hormon could not be identically repeated since the source of the DNA in humans varied with the individual. In general, n component should be defined in functional terms in this field of technology. As to the inventive step the Examinin Division construed the experimental disclosure of the Polisky paper (1) as sufficiently encouraging to develop th expression of heterologous DNA in bacteria. It was said to be known from the reference that for transcription t occur the DNA must be inserted in the correct reading frame. This was the position in spite of the fact that the DN used did not or could not lead to a translatable protein. Moreover, (cf. page 3904, last paragraph, second sentence the article envisaged and suggested examination of the possibility of translating normally occurring eukaryotic DN sequences, and foresaw that extensive translation of a functional heterologous polypeptide might occur. It was als suggested by the Examining Division that the claim was rather an expression of an obvious problem and there was n invention in that.

III. The Appellant submitted a Notice of Appeal against the decision, together with payment of the fee on 13 Septembe 1985 and filed a Statement of Grounds on 22 November 1985. Some observations were filed under Article 115 EPC by third party on 15 September 1986 citing an article by Selker et al., J. Bacteriology, 1977, 129, 388-394 (2) agains the patentability of the claimed subject-matter. The Appellant was invited by the Board under Article 110(2) EPC t file comments on the observations. The comments were then received on 30 December 1986. The Board thereafte issued a Communication on the substantial issues of the case on 2 June 1987, raising in particular the role and th provision of the regulon as one of the critical features, and the Appellant filed a reply on 28 August 1987 including additional sets of claims filed as auxiliary requests

IV. An oral hearing was held on 26 and 27 January 1988. During the course of the hearing a new request with 16 claim was submitted on behalf of the Appellant to replace all earlier main and auxiliary requests. Claims 1 and 9 to 1 were worded as follows: "1. A recombinant plasmid suited for transformation of a bacterial host wherein the plasmi comprises a homologous regulon, heterologous DNA, and one or more termination codon(s), the heterologous DN encoding a desired functional heterologous polypeptide or intermediate therefor which is not degraded by endogenou proteolytic enzymes, said DNA being positioned in proper reading frame with said homologous regulon between sai regulon and the termination codon(s), whereby on translation of the transcription product of the heterologous DNA in suitable bacterium, the resulting expression product is said desired functional polypeptide or intermediate therefo in recoverable form.

9. A process for the production of a recombinant plasmid as defined in any one of the preceding claims which comprise treating a length of double stranded DNA comprising an intact replicon and in sequence (a) a regulon for controllin transcription and translation in a bacterial host and (b) a restriction endonuclease recognition site, with a suitabl restriction endonuclease to form a DNA fragment that comprises the replicon and the regulon, and ligating thereto i proper reading frame with said regulon a heterologous DNA coding for a functional heterologous polypeptide o intermediate therefor which is not degraded by endogenous proteolytic enzymes, said heterologous DNA having terminal nucleotide grouping which is ligatable to said DNA fragment, to give said recombinant plasmid.

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10. A bacterium transformed with a recombinant plasmid according to any one of Claims 1 to 8.

11. A bacterial culture comprising transformed bacteria according to Claim 10

12. A process for the bacterial production of a functional heterologous polypeptide or intermediate therefo comprising growing a bacterial culture as defined in Claim 11 to bring about expression of said polypeptide o intermediate.

13. A process according to Claim 12 for producing an immunogenic substance comprising a polypeptide hapten comprising: (a) providing a recombinant plasmid containing a homologous regulon, and in proper reading fram therewith, a heterologous DNA sequence encoding the hapten, a DNA sequence encoding a second amino aci sequence sufficient in size to render the product of DNA expression immunogenic and one or more termination codons (b) growing a bacterium transformed with the recombinant plasmid, occasioning expression of a conjugate polypeptid consisting essentially of the amino acid sequence of the hapten and the second amino acid sequence; and (c) testin the conjugate polypeptide for its ability to raise antibodies against said hapten."

V. The Appellant submitted in the proceedings and at the oral hearing substantially the following arguments: (a) Th assessment of the contribution to the art in comparison with the Polisky (1) reference must be kept in mind whe considering the question of Article 84 EPC. According to the "Protocol on the Interpretation of Article 69 of th Convention", the construction of the protection conferred by the European patent must be interpreted as combining fair protection for the patentee with a reasonable degree of certainty for the third parties. Thus, the character o the advance should have a bearing on questions of scope and **support**. (b) The inventive step must be assesse through the eyes of the hypothetical ordinary person skilled in the art and not on the basis of a researcher such a experts like Polisky and his co-workers, who were very perceptive, ingenious and imaginative. Yet they only went a far as explaining transcription in their article except for a short passage which was very speculative. (c Publications before the Polisky paper (1) showed no success with transcription when inserted DNA, originating from frog, had been under heterologous control, and therefore the task for Polisky and his co-workers was to see whethe homologous control would be successful instead. Even if there was some incidental translation involved, there was, t others, no way of knowing what happened exactly since neither the inserted fragment nor the result was sequenced. I any case, the heterologous DNA was only inserted for transcription since it was only intended to yield basicall untranslatable ribosomal RNA sequences. (d) There were some assumptions in the cited paper as to partial incidenta translations of the DNA sequences in view of a distinctive ß-galactosidase sequence in the plasmid (hereinafte ß-gal), but the repetition of the results was not possible with further ribosomal DNA fragments from the frog Notwithstanding this, in the final paragraph the paper speculated on the possibility of expressing a "functiona eukaryotic polypeptide" but this was envisaged to be carried out with a heterologous ribosomal binding site and no with a homologous regulon for the purpose. The hypothetical use of the latter was clearly seen as yielding only covalent hybrid with ß-gal, containing only a sequence corresponding to the heterologous DNA until the firs "nonsense", i.e. stop-codon. No appreciation of the need for an in-phase relationship appeared in the paper which i considered to be the closest state of the art. The Examining Division made a technical error in deducing fro Polisky's experimental work that a skilled reader would be taught anything about the need for a correct readin frame. (e) As to the question of sufficiency of disclosure and the related question of clarity and **support** for th claims under Articles 83 and 84 EPC, the host of publications following the invention showed its genera applicability and value as a pioneer invention. Functional terms like suitable bacteria were governed by the fact tha a homologous regulon only works in bacteria where they were ordinarily endogenous. There was no reason to assum that the invention would be unworkable under the suggested circumstances. Insufficiency should be a matter o evidence showing that failure was inevitable even under a bona fide effort. (f) It was basically enough to show on way of carrying out the invention. The invention was not identical with its elements since the variants of it constituents could be freely substituted for each other. None of its elements which might have turned out to b inventive in the future was claimed per se. The method had virtually infinite applicability to provide any polypeptid which is large enough. No direct utility of the products and intermediates prepared by the invention was required. (g With regard to the question of providing the regulon in the correct in-phase position, i.e. proper reading frame, th ATG starting code must either be immediately followed by the inserted gene or there must be multiples of three as t the number of nucleotides between this and the relevant gene in case of conjugated fusion nucleotides. Th specification disclosed how to add single nucleotides and there were references in the literature showing how t adjust the sequence to any desired length. A number of control regions were available at the priority date and th knowledge of restriction sites enabled appropriate tailoring in this respect. The outcome of the digestion of plac DNA, utilised in the application, was well known at the priority date of the application.

VI. The Appellant requested that the decision under appeal be set aside and that the patent be granted on the basis o the description and Claims 1 to 16 as submitted during the oral proceedings, with the drawings as originally filed

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Reasons for the Decision

1.The appeal complies with Articles 106 to 108 and Rule 64 EPC and is, therefore, admissible.

2.Amendments. The amendments which are incorporated in the present claims are not such that the application contain subject-matter which extends beyond the content of the application as filed (Article 123(2) EPC). Furthermore, suc claims are supported by the description (Article 84 EPC)

2.1. In particular, the feature "termination codon(s)" added to the main claim was taken from page 2, lines 3-5. Th phrase: "... said DNA being positioned ... between said regulon and the termination codons ..." follows from th initiating role of the regulon and termination role of the codons, from page 2, lines 5-7, as well as from th examples illustrating this. The other features are either directly taken from original Claim 1 or **implied** by an therefore derived from the whole disclosure as such. Thus, the phrase "desired functional" qualifying the ter "heterologous polypeptide" is explanatory and can, in view of the product to be obtained, only mean the exac correspondence between the DNA code and the result of the expression. It distinguishes the claimed plasmid from thos processes which might only provide undesired (junk) proteins. The term "intermediates" is accordingly indicative o polypeptides which are used in order to obtain the desired ones, for instance via a larger protein as exemplified i the disclosure.

2.2. As to the limitation of the polypeptide products to be obtained with the plasmid, to those which are "no degraded by proteolytic enzymes", this is based on the statements from page 25, line 26 to page 26, line 3, whic provide a general teaching in this respect to the skilled person. Subject-matter which was not capable of solving th technical problem was thereby excluded from the scope of the claim. In spite of the fact that the plasmid containe the desired somatostatin DNA fragment properly inserted (cf. page 25, lines 21-25) no expressed product could b detected. The amendment also removes the ground for objection under Rule 27(1)(f) EPC (cf. impugned decision, pag 12) which suggested that neither somatostatin nor the insulin chains had been expressed and recovered directly. Th claim is now confined to circumstances where no degradation occurs and recovery is possible.

2.3. The rest of the claims in the request either correspond to original claims or rely on the disclosure. Of th latter group, Claim 3 is **implied** by page 6, lines 1 and 2; Claim 4 is based on page 6, lines 4 and 5; Claim 7 o page 10, lines 23 and 24; Claim 9, as far as the additional features are concerned on page 3, line 24 and page 5 lines 8-15; Claim 10 on page 17, lines 23-24; Claim 11 on page 17, lines 36 et seq; and Claim 12 on page 40, line 2 to page 41, line 22, as well as on the description as a whole

2.4. The addition of new dependent Claims 13 to 16 is based on the disclosure in the description (page 13, line 22 t page 14, line 12) as to the possibility of providing immunogenic conjuncts with "hapten" polypeptides, and on th actual preparation of such conjuncts with somatostatin in the Examples (pages 26 to 30 and page 33, lines 32-35). I particular, the requirement that the second amino acid sequence should be sufficient in size to render the produc immunogenic is based on lines 29 and 30 of page 13, and the respective references to radioimmune activity of th somatostatin-conjunct **support** the immunogenic character of the products and the corresponding need for a testin step as specified in Claim 13, in many instances. All claims are therefore acceptable as being supported by th disclosure and complying in this formal respect with Articles 84 and 123(2) EPC. The consequential amendments of th specification presented at the oral hearing, containing also corrections of obvious typing errors, are allowable.

3.Sufficiency and **support** (Articles 83 and 84 EPC). Objections on these grounds relate to the non-availability o some embodiments in certain circumstances and the alleged necessity of reproducing each and every embodiment of th invention. In addition, broad functional claiming may embrace the preparation of future products, the patentabilit of which being thereby prejudiced. Such objections under Article 83 EPC would, if justified, lead in this case t further objections under Article 84 EPC on the ground that the claims are, consequently, not properly supported i their scope. The various problems which were raised in the impugned decision and others which are recognised by th Board will hereinafter be discussed separately.

3.1. Components of the future

3.1.1 Recombinant plasmids embrace, as components, various regulons which have not yet been provided and may, on day, represent inventions on the basis of some merit of their own. The same applies to the basic plasmid, which ha been modified to possess the characteristics of the claim. The original plasmid might have complex structures to b developed in the future. Bacteria transformed with the claimed plasmids embrace mutant or modified forms not ye known. According to the Examining Division this situation contradicts the suggested requirement that all embodiment

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within the claims should be reproducible at will by the skilled person without having to make an invention.

3.1.2 There is, however, in the opinion of the Board, no such requirement in the European Patent Convention, nor i such principle established in normal patent practice within the Contracting States. The suggested features in th claims are essentially functional terms in this particular context, in spite of structural connotations, and ma cover an unlimited number of possibilities. It follows that the features may generically embrace the use of unknow or not yet envisaged possibilities, including specific variants which might be provided or invented in the future This Board concurs with the decision of another Board (T 68/85 -3.3.1., "Synergistic herbicides", OJ EPO 1987, 228 in which the possibility of using functional terminology in claims was approved if "such features cannot otherwise b defined more precisely without restricting the scope of the invention" and their reduction to practice was not a undue burden. The Board sees no valid reason why this should not be equally true for the field of biotechnology as i other fields of technology. In appropriate cases, such as the present, it is only possible to define the inventio (the matter for which protection is sought - Article 84 EPC) in a way which gives a fair protection having regard t the nature of the invention which has been described, by using functional terminology in the claims.

3.1.3 What is also important in the present case is the irrelevancy of the particular choice of a variant within th functional terms "bacteria", "regulon" or "plasmid". It is not just that some result within the range of polypeptide is obtained in each case but it is the same polypeptide which is expressed, independent of the choice of these means A term of this kind must, of course, be clear and enable the skilled person to find suitable specimens without undu difficulty. In the present application enough choice is available, although some vehicles and hosts are preferred fo practical reasons.

3.1.4 The objection raised against the terms "plasmid" and "bacteria" that they are too broad since some of them rel on yet unavailable entities is untenable. The Board is of the opinion that this is quite normal practice in man technical fields where terms as "carriers", "resilient means", or "amplifying means" are commonplace and embrace ne components, be they inventive or not. This is not to mention that very often the generic indication of a kind of a article in the claim is followed by the non-exclusive term "comprising" and the characteristics of modifyin features, leaving completely open the actual features of the rest of the article, apart from the necessity that it functioning should be as expected.

3.1.5 The above examples show that the need for a fair protection governs both the considerations of the scope o claims and of the requirements for sufficient disclosure. Unless variants of components are also embraced in th claims, which are, now or later on, equally suitable to achieve the same effect in a manner which could not have bee envisaged without the invention, the protection provided by the patent would be ineffectual. Thus it is the view o the Board that an invention is sufficiently disclosed if at least one way is clearly indicated enabling the skille person to carry out the invention. Consequently, any non-availability of some particular variants of a functionall defined component feature of the invention is immaterial to sufficiency as long as there are suitable variants know to the skilled person through the disclosure or common general knowledge, which provide the same effect for th invention. The disclosure need not include specific instructions as to how all possible component variants within th functional definition should be obtained.

3.1.6 The Examining Division's tentative suggestion that such terms should be restricted to those available in the ar has no basis in existing law. Unless broad, yet proper terminology is allowable, subsequent investigations by thir parties might be encouraged to concentrate on finding alternatives outside the claims instead of trying to pursu progress through dependent inventions. The lack of recognition of the full significance and the interdependency o technical contributions could adversely affect progress in the area of microbiology and biochemistry.

3.1.7 In view of the above, it is also irrelevant that some of the variants of bacterial strains or regulons migh only exist in private collections or can only be found in locations or derived from sources which are inaccessible o were only transiently available to the public. As long as there are means available for performing the invention such exceptional circumstances cannot counteract the possibility that the invention can be carried out.

3.2. Inoperable components.

3.2.1 Whilst the Board is satisfied that there are sufficient choices of bacteria available, and that there might b more suggested in the future, the question of non-operability of some bacterial variants may arise. Whilst there i so far no reason to doubt that homologous regulons would also reliably work in the microbial environment of thei origin, the term "bacteria" might include inherently inoperable species or variants. However, the main claim refer to a "suitable bacterium", and Claim 10 to a bacterium transformed with the claimed plasmids, which in any case impl to the skilled reader that this should be a bacterium in which the homologous regulon is "at home" and can b operative. In addition, the bacteria to be used may be modified to enhance their suitability. Whilst such express or implied functional limitations are acceptable in the present application, since the applicability of the method t

any kinds or most species of bacteria has not been effectively challenged, this may not be the case if the skille person cannot easily find his way to put the invention into effect, for instance with the specially recommende bacteria or plasmids. It is, therefore, also the view of the Board that the unsuitability of some unspecifie particular variants of a functionally defined component feature of the invention is immaterial as long as there ar suitable variants known to the skilled person through the disclosure or common general knowledge which provide th same effect for the invention.

3.2.2 The burden of finding workable candidates is related to the relevance of such functional feature to th inventive step, i.e. its essentiality to the quality or quantity of the effect obtained and thereby to it distinguishing power against the relevant prior art. Some features may contribute to the core of the invention an others only assist their use, and the skilled person might, therefore, be in a more difficult or an easier positio to find suitable choices. For instance, bacteria themselves only enter a further, dependent aspect of the invention (Claim

10). In view of the supplementary role of bacteria as housings for expression and of the general character of the ter representing a further restriction of the claim in question, it would also be unreasonable to impose an additiona limitation to its scope for the reason alone that some of the specimen may not be suitable at all.

3.3. Details influencing the result

3.3.1 The above situation with respect to possible ineffectual bacterial variants embraced together with effectiv ones, must, however, be carefully distinguished from failures to obtain certain kinds of effects at all, i.e. specific polypeptide product in our case. The range of DNA inserts was restricted in the proceedings before the Boar to those which provide directly recoverable polypeptides, i.e. those which are "not degraded by endogenou proteolytic enzymes". This limitation is not only formally proper (cf. Item 2.2) but also necessary since the skille person had no other method available to him at all to obtain directly any degradable polypeptides. The only rout leading him to the degradable small polypeptides was indirectly through a cleavable large product, which is a proces claimed in a co-pending application and also described in the examples of the present specification, being no available in the literature. This involves matter outside and independent of the present claims, which only relate t directly obtainable undegradable, e.g. large polypeptides. The limitation is therefore necessary to eliminat effectless versions of the invention, and to define the subject-matter of the invention precisely.

3.3.2 Whilst there is no doubt left that the required polypeptides within the scope of the claims would always b obtainable in a recoverable form whenever the corresponding DNA insert coding for it is duly incorporated in th plasmid, i.e. "identical" reproduction is obtainable, some natural sources for particular DNA's might only b temporarily available. The impugned decision mentions the case of unique human hormones, where the source for th mRNA, and thereby the cDNA, may die or become otherwise non-available. Thus, exactly the same polypeptide can onl be provided in the suggested manner if an exactly corresponding DNA insert is available. The Board has held in th decision of the case T 281/86, ("Preprothaumatin", 27 January 1988, published in OJ EPO 1989,202) that there is n requirement under Article 83 EPC to the effect that a specific example of the process claim must be exactl repeatable. Variations in the constitution of an agent used in a process are immaterial to the sufficiency of th disclosure provided the claimed process reliably leads to the desired product" (cf. page 8, emphasis added). It i important to know that in that case the set of products was limited and all were obtainable as desired. The presen application is, however, not concerned with the problem of obtaining a finite set of particular products, as in th cited decision. The character of the invention this time is one of general methodology which is fully applicable wit any starting material, and is, as it was already stated, also independent from, the known, trivial, or inventiv character of the end-products. The transformed bacteria, as well as the claimed plasmids are agents and geneti precursors in a process of transformation, expression and recovery leading to the programmed products, and as long a the system works reliably at every stage there is no obligation to exclude future starting materials.

3.3.3 It is therefore the view of the Board that generally applicable biological processes are not insufficientl described for the sole reason that some starting materials or genetic precursors therefor, e.g. a particular DNA or plasmid, are not readily available to obtain each and every variant of the expected result of the invention, e.g. th product, provided the process as such is reproducible. In chemistry, widely applicable chemical reactions have bee claimable without restrictions to irrelevant structural details. The same should be applicable to biochemical processes.

3.3.4 It is also relevant to the generality of broadly claimed new methods that the generic terminology migh inevitably imply novel products, e.g. novel proteins. The Examining Division erred in its assumption that suc coverage embracing the "first chemical synthesis" of such a product, would prejudice the patentability of the same presumably for lack of novelty. Apart from the maxim that the general does not normally imply the particular especially if the former is not construed to disclose its members in an individualised manner, the idea that suc methods cannot broadly relate to dependent future inventions and developments must be rejected in principle as bein contrary to established practice. In chemistry, widely applicable reactions were claimable, notwithstanding the nove

or known character of the products. If the implications suggested by the Examining Division were correct, there woul be no room for selection inventions.

3.4. The generality of the regulon.

3.4.1 The provision of a homologous regulon in the plasmid in conjunction with a heterologous DNA insert in a certai arrangement is an essential and important characteristic of the claimed subject-matter. The Board itself raised the question of sufficiency with regard to such homologous regulons which are to be present in a proper reading fram with the inserted DNA sequence. The structure of regulons, having been part of the expression system of the plasmi candidate associated with certain bacteria, is either already known or can be established by appropriate analysis. A example had used appropriately modified plac 5 DNA by digestion with the restriction enzyme Hae III to remove most o the ß-gal sequences (cf. page 15, lines 23 to 30, page 22, line 27 to page 23, line 21), which ascertained that th regulon incorporated was in- phase with the DNA insert. In further examples (page 27, line 1 et seq. and page 39, lin 30 to page 40, line 18), an inserted fragment of the same plac 5 again contained the lac control region, together wit most of the ß-gal structural desired polypeptide incorporated (insulin A or B sequences).

3.4.2 The general description explains the character of the control elements including the preferred lac-operato system which may also deliver, if desired, the ß- gal component. Other control systems are also recommended (page 14 line 30 et seq.). To **support** the contention that it was known from common general knowledge how to adjust th sequences by adding or deleting nucleotides, the Appellant referred to the publication of Scheller et al, Science 1976, 196, 177-180. This enables the adjustment of a DNA to any length so that a proper reading frame is obtained References were also made in this respect to Bahl et al, Gene, 1976, 1, 81-91, and to Heyneker et al, Nature, 1976 263, 748, (cf. specification, page 20, line 25). The latter, of course, established the manner of constructing th lac-operator used in the application.

3.4.3 In the absence of evidence to the contrary, the Board accepts that the above publications which were no expressly referred to in the specification reflected common general knowledge. At that time, when there was considerable effort everywhere to achieve success in the manipulation of plasmids and genes, the cited paper represented important disclosures as to how to do this and change the length of DNA's at will. Hence, it must b assumed that everybody concerned became aware of their teaching. Professor Glover, who had been introduced to th Board as an expert with considerable experience in the genetic field, and who assisted the Board on behalf of th Appellant by answering technical questions at the oral proceedings, confirmed that the cited articles had the sai character, i.e. could be construed as having been part of common general knowledge for a molecular biologist.

3.4.4 Professor Glover also confirmed that the presence of the initiating codon ATG straight in front of the firs codon of the desired protein did not itself yet guarantee that the system was in-phase for expression and that th binding site for ribosome and optionally other sites assisting the initiation of the expression, such as promoter-operator system to switch the process off and on, have to be appropriately provided to make sure that th expression system works in a proper reading frame in the circumstances. It was also well known how to equip th opened plasmid and the DNA insert with the necessary sticky ends for ligation. Preferably the pairs' sticky ends woul be different so as to avoid insertion in the wrong orientation.

3.4.5 The Board recognises that the plasmid would also be equipped with a suitable promoter site for transcription and replicon sequences to ensure that the plasmid is capable of replicating. Since cloning is an essential step i the preparation of the plasmid, the standard sequences for resistance to tetracycline and ampicillin are als envisaged, although other means may also be incorporated to improve performance. These are therefore **implied** features clearly expected by the skilled person to be there in order to satisfy the meaningfu functional limitation in the claims that the recombinant plasmid is "suited for transformation of a bacterial hos ... whereby on translation ... the product is said desired polypeptide ..." It is, therefore, in these circumstances unnecessary to burden the claims to plasmids with the express listing of **implie** features.

3.4.6 The Board has no reason to doubt that the necessary incorporations in the plasmid can be made on the basis o the examples and general explanations in the disclosure, to provide the regulon and other constituent sequences in reading frame and in the form of functional capability, and that the skilled person was also in a position to do thi differently, as for instance with other regulons known to him. As to the questions relating to sufficiency, it can b concluded that the disclosure is adequate and sufficient in the circumstances and there is enough information t apply the subject-matter generally for the stated purposes. The requirement of Article 83 EPC is thereby satisfied It follows that the terms of the claims are, in these respects, also adequately clear and supported in their scop (Article 84 EPC)

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4.The problem and the solution

4.1. The claimed subject-matter relates to the expression of desired heterologous polypeptides in a bacterial hos under the control of a homologous regulon, in a recoverable form. There was no reference cited from the state of th art where this effect had been achieved before. In the view of the Board the closest state of the art is represente by Polisky (1), describing the construction of a plasmid, suitable for the transformation of E. coli bacteria, whic has a bacterial regulon and heterologous DNA fragment inserted into it, coding for a ribosomal RNA sequence (rRNA) The sequence did not code for a translatable polypeptide at all, but for the rRNA which was to become part of ribosome (cf. page 3902, right column under the heading "Expression of eukaryotic DNA under lac- control). Th article, however, speculates about the possible use of the idea for producing "eukaryotic gene products in bacteria in general (last sentence of abstract and at the end of the discussion, page 3904, left column, last paragraph).

4.2. The speculative statements in the reference could be construed as a tentative reference to a technical proble arising from the disclosure. This was to provide, at last, the expression of specific heterologous polypeptides i bacteria as useful products, corresponding exactly,to the DNA insert. Several literature references before th priority date suggest that this was the problem of biochemistry and it was not therefore "invented" with the presen case. This, of course, means that the recognition of the problem alone cannot contribute to the inventive step. I was even announced by the inventor at a Congress and subsequently by a technical weekly before the priority date o the present application that somatostatin had been expressed, without disclosing how this was achieved (26t International Congress of Pure and Applied Chemistry, Abstracts, Session I, Tokyo, Japan, September 4-10, 1977 (3 and C & EN, 1977, No. 7 (4)). The solution of the problem, claimed in the present application, requires that th heterologous DNA insert should be in a proper reading frame with the homologous regulon in the plasmid for th transformation of a suitable bacterial host and that the DNA must code for a polypeptide not degradable b proteolytic enzymes, for instance because of its size. In other words, contrary to the expectations arising i consequence of the announcements, there is no direct route provided by the invention to somatostatin and the lik small polypeptides, but to larger proteinaceous entities, although the specification also demonstrates how th smaller ones could, though only partly, through the invention, be indirectly approached by making also use of further invention, separately claimed in a co-pending application.

4.3. Whilst the specification admits that no somatostatin could be detected at all in spite of proper insertion of th gene (cf. page 25, lines 21 to page 26, line 3), the disclosure attributed the failure to the intracellula degradation by proteolytic enzymes. The inventors in the case tried a different approach through a precursor whic was sufficiently large as a polypeptide to resist degradation and thereby provided a route to such larger entities The test showed that a precursor protein, incorporating also somatostatin gene, was indeed obtained which yielde somatostatin after cleavage with cyanogen bromide. After standard enrichment and separation processes, pur somatostatin was obtained (cf. page 33, line 14 to page 34, line 10). Similar success was achieved with the insertio of genes coding for chains A and B of human insulin. This is then again credible evidence that the large hybrid incorporating an insulin A or B chain, was duly expressed, proving that the translation was indeed in-phase. Afte cleavage, the products showed that, for instance, the insulin A chain could be isolated and identified by the correc amino acid composition (cf. page 41, lines 16-22). It was stated on behalf of the Appellant that the homologou character of the added protein was irrelevant to the results and that the system would work, as claimed, for an desired large protein directly. On this basis the Board has therefore come to the conclusion that polypeptides larg enough to be unaffected by proteolytic enzymes could be expressed at will with the disclosed method in a recoverabl form.

5.Novelty. In view of the fact that no cited document discloses either the plasmids containing expressibl heterologous, e.g. truly eukaryotic DNA, or the instruction to produce polypeptides of the kind which resists th action of degrading enzymes by using a homologous regulon which is in a reading frame with the DNA, the novelty o the application is not in doubt and has not been an issue in the proceedings.

6.Inventive step.

6.1. As already stated, the closest state of the art is (1), since this already uses a homologous regulon in E. col in combination with a heterologous DNA and proceeds as far as transcription of the DNA in order to provide a rRN sequence. The task then was to demonstrate this possibility, and this required no regulon in-phase with the DNA since transcription proceeds through each nucleotide one by one, under the control of a promoter. The Examinin Division, therefore, cannot be followed in their inference that "from the Polisky reference it was known that th heterologous DNA must be inserted in a correct reading frame to be expressed". The rRNA involved in the articl would, at the best, be expected to produce only small fragments of polypeptides in an incidental manner, if exposed t an expression machinery, since such kinds of RNAs have many stop codons to terminate any incidental expression

6.2. Nevertheless, the paper speculates in the direction of expressing an eukaryotic DNA in bacteria, and the questio

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arises what basis is given in the paper to contemplate the necessary modifications which we now know are essential fo success, and where such changes could come from, according to relevant knowledge at the time of the priority date Before Polisky (1), several workers introduced eukaryotic DNA sequences into plasmids but there was no evidence tha their eukaryotic promoter sequences were correctly recognised by bacterial RNA polymerases (Ibid, first paragraph) The aim was therefore to test the transcription under the control of the bacteria's own promoter. The use of fragment of a frog's (Xenopus laevis) DNA coding only for a transcription into a rRNA, had been explored and th results showed that the transcription was successful and even increased under the control of the lac-operon.

6.3. Since there was no hint in the paper that any sequencing had been done, there would have been no chance o detecting whether or not any translation of the ß-gal of the plasmid had also contained added polypeptide sequence from an (incidental) translation of any part of the ribosomal RNA sequence, let alone whether the translation wa correct. Nevertheless, (1) suggested that the ß-gal had lower enzymatic activity and was distinguishable from th normal, wild-type of enzyme (cf. page 3904, left column, second paragraph). There is also a conjecture in (1) that a in-phase read through translation might have occurred, based on a part of the RNA fragment from the frog DN (presumably until the first stop-codon) if the "normal translational stop signals for ß-gal are missing" in th plasmid (page 3904, left column, second paragraph, lines 12-14). There is no explanation why this should be the case and the only **support** for the hypothesis is that induced cultures indicated somewhat higher level of highe molecular weight ß-gal than what is normally the case with the wild-type ß-gal. The paper goes on expressing th belief that this might have happened leading to a fused polypeptide covalently linked to ß-gal (page 3904, lef column, second paragraph, lines 20-22). There is, of course, no information in the article about the exact position o the EcoR 1 cleaving site and thereby about the position of the reading frame, since there was no need for suc function within the framework of the experiments.

6.4. The authors of the paper themselves investigated further the extent of readthrough translation, and reported som other experiments involving insertion of other Xenopus DNA fragments of the ribosomal kind, but could not then detec any inducible readthrough transcription in either orientation (page 3904, left column, second full paragraph). Ther is no explanation as to the reason of failure in these further experiments.

6.5. With this background of facts in mind the authors finish the paper by suggesting the possibility of examining th expression of "normally translated eukaryotic sequences". It is immediately stated that a ribosome binding site whic was brought in with the DNA, i.e. an heterologous regulon system, might allow an extensive translation of functional eukaryotic polypeptide, i.e. the desired product of a particular gene. In contrast, the next sentenc refers to the expression of "a peptide covalently linked to ß-gal" in the absence of such "independent ribosom binding site". The latter statement must be understood to mean the abandonment of the first proposed eukaryoti ribosome binding site since this would be between the ß-gal sequence and the polypeptide sequence. The price for thi is a covalently, i.e. inextricably linked hybrid of ß-gal with "a peptide", which is only a fragment of the gene code for, since the last sentence reveals that the readthrough translation would only go until the first "nonsense" codo is reached.

6.6. Thus, the paper itself envisages no reading frame since this would have led to "nonsense" codon. This and th failures to obtain even partial readthrough translations reliably, in spite of expectations, represent no rea signposting towards the invention, which aims at obtaining the whole functional polypeptide and not a wrongl translated fragment of it, irreversibly bound to ß-gal. If anything, the paper preferably points towards heterologous regulon and not to a homologous one, and when it allows the homologous one to function it accepts that covalently bonded nonsense hybrid would be formed.

6.7. There is no hint how to pursue the second, less attractive line to obtain nevertheless a desired correct product As we now know, the skilled person would have needed the proper reading frame and a minimum size for the immediat product. In the absence of any appreciation in the paper about the necessity of employing an in-phase arrangemen consciously, the skilled person facing the problem of obtaining the right polypeptide at will, should have looke around in the state of the art to find some means which would provide the desired effect. The puzzle for th researcher would have been intensified by the reports in (3) and (4) that it was somehow possible to expres somatostatin. Since there was no suggestion anywhere how the aim can be achieved, i.e. any enabling disclosure o success in this respect, and the complete silence in the literature about any link between a homologous regulon and heterologous DNA in a reading phase, this modification, which is the first essential characteristic of the invention could not have been straightforward and obvious with regard to the ambitious expectations.

6.8. In spite of the fact that Polisky points away from the present invention and towards a different strategy t achieve proper expression of the whole DNA gene, the skilled person might have been keen and anxious to solve hi problem already recognised in the literature as the problem of biochemistry. If by some inspiration he had hoped t improve the less promising second suggestion in the paper and to try the use of the bacterium's own regulon and eve

to do something about proper reading frame, he would have been likely to choose a relatively simple DNA to produce small polypeptide for insertion in order to test the idea. This is because the synthesis of small genes wa relatively simple and so was the identification of corresponding polypeptides in the result. On the other hand, th synthetic preparation of large genes was cumbersome or impossible and it was difficult to obtain, identify and revers transcribe the information from natural mRNA to cDNA for insertion, and then test the result. This would have been a enormous task and unless there was full knowledge as to the structure of the insert and the resulting polypeptide, th experiment would be useless to demonstrate whether or not the problem has been solved. Thus, a modest start with small polypeptide, possibly encouraged by the report that somatostatin was successfully translated, would have bee his aim, which should, as we now know, have led him into a failure in view of the destruction of the result b proteolytic enzymes.

6.9. There would have been no way to know for sure at that time as to what went wrong and there was no good reason t assume that the same experiment would necessarily work well with a much larger protein. It must be emphasised tha there is no suggestion in the Polisky paper to the effect that the avoidance of degradation is of any significance i relation to solving the given problem. The skilled person would have been discouraged to embark upon the exercise o testing a larger entity and would have had no knowledge from anywhere about combining a small entity with a cleavabl ballast as a way out of his dilemma. Thus, envisaging the second essential characteristic criterion of the invention the necessity of working with polypeptides which are not readily degraded was not obvious to the skilled person either.

6.10. Even if the above thought experiment is disregarded, it remains relevant that the distinction between large an small polypeptides is critical for success and failure, and that this was nowhere available in the state of the art The Board also recognises that its conclusion about the inventive character of the claimed subject-matter i confirmed by the circumstances in the art, before and after the priority date. There have been many articles in th literature before the priority date involving the insertion of DNA into a plasmid, yet none of them obtained result which would have showed that bacteria can be made to manufacture what the programme prescribes, which is exactl the polypeptide corresponding to an insert. There have been many shots, some of them valuable contributions to ou knowledge and to further developments, but none of them hitting the target (cf. submissions from the Appellant date 16 July 1981). In contrast to this, there are more than one hundred publications and dozens of patent applications which make use of the invention claimed, after it became public (cf. the Kleid affidavit filed 6 February 1985). Thi sudden cascade of applications after a period when everybody must have strongly desired the breakthrough, should b taken as a confirmation of inventive step, and even as a sign of pioneering significance.

6.11. The copy of an article by Selker (2), which had been submitted in the proceedings before the Board in Septembe 1986, under Article 115(1) EPC by a third party, was examined in the light of observations and comments from th Appellant. This was done in order to establish whether or not the citation is prima facie closer art than (1). Th Board has come to the conclusion that this paper is not clearly a more relevant state of the art than what ha already been under consideration.

6.12. The Board recognises the inventive step and the broad applicability of the plasmids claimed in the presen application. This indeed necessitated the careful assessment of the scope of the subject-matter claimed in order t give a fair protection to the patentee. Unless the features of the claim are construed as proper in embracing presen and future uses of the invention, and in fact all conceivable uses of the inventive idea, the patent system woul fail to serve its purpose. The non-obviousness of the plasmids also imparts an inventive step to the other claime subject-matters relating to their preparation and to their use for making polypeptides and immunogenic substances.

ORDER

For these reasons, it is decided that:

1. The decision under appeal is set aside.

2. The patent is granted on the basis of description and the Claims 1 to 16, as submitted during the oral proceeding with the drawings as originally filed